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Studies on Pyrimidinylpyrazoles. IV.[1,2] Pharmacological Activities of 1-(4-Methoxy-6-methyl-2-pyrimidinyl)-3-methyl-5-methoxypyrazole[3] and Its Related Compounds

Yasuo Oshima, Takeshi Akimoto, Wataru Tsukada, Terukiyo Yamasaki, Kunio Yamaguchi and Hiroshi Kojima

Central Research Laboratory, Daiichi Seiyaku Co., Ltd.[4]

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In order to search for a highly potent drug, 1- and 2-pyrimidinylpyrazole derivatives have been examined for analgesic, antipyretic and anti-inflammatory activities. Among the 44 derivatives examined in this series, all of the compounds which are equal to or more potent than aminopyrine in analgesic activity were found to belong to 1-pyrimidinylpyrazole type, while all of 2-pyrimidinylpyrazole derivatives were extremely less active than aminopyrine in analgesic effect. Three compounds which possess a methoxyl group in the 5-position of the pyrazole moiety were found to have especially high potency in this series. It has been found that 1-(4-methoxy-6-methyl-2-pyrimidinyl)-3-methyl-5-methoxypyrazole is the most potent compound having analgesic and anti-inflammatory effects.

It is well known that pyrazolone derivatives have analgesic and antipyretic activities. Although some of the compounds have been widely used clinically, there have been few reports on pyrimidinylpyrazole derivatives. As described previously,[5] 1- and 2-pyrimidinylpyrazole derivatives were synthesized in this laboratory to find a highly potent drug.

The present study was undertaken to examine analgesic, antipyretic and anti-inflammatory activities of these compounds.

Materials and Methods

Test Compounds——The test compounds used are listed in Tables I, II and III. Some compounds were dissolved in neutral, acid or alkaline water, and the others, being poorly soluble in such water, were suspended in 10% acasia for administration.

Analgesic Activities——a) Pressure Method: A method described by Takagi, *et al.*[6] was used for the analgesic test. Male mice of ddN-JCL strain, weighing from 14 to 30 g, were used in groups of 10 animals each.

The pain threshold was measured 2 times before and 5 times after administration of the compound at 15-min intervals. The potency ratio was calculated by the four-point assay.

b) Electric Stimulation Method: The analgesic activities of some of the compounds were examined according to the method described by Ozawa.[7] The base of a mouse tail was stimulated with rectangular pulses of 25 V and 40 msec duration and 1 cps frequency.

Male mice of ddN-JCL strain (14—25 g) which squeaked on three or less stimulations were selected for this experiment. The analgesic effect was determined as positive when the animals failed to squeak even on five or more stimulations after administration of test compounds. The activity was expressed

1) Part III: M. Sano, I. Itoh, Y. Nakai and T. Naito, *Chem. Pharm. Bull.* (Tokyo), **17**, 1485 (1969).
2) This work was presented at the 88th Annual Meeting of Pharmaceutical Society of Japan, Tokyo, April 1968.
3) Methopyrimazole has been proposed as generic name for this compound.
4) Location: *Minamifunabori-cho, Edogawa-ku, Tokyo.*
5) T. Naito, T. Yoshikawa, S. Kitahara and N. Aoki, *Chem. Pharm. Bull.* (Tokyo), **17**, 1467 (1969).
6) K. Takagi, T. Kameyama and K. Yano, *Yakugaku Zasshi*, **78**, 553, (1958).
7) H. Ozawa, *Folia Pharmacol. Japon.*, **49**, 67, (1953).

in terms of ED_{50} calculated according to the Litchfield and Wilcoxon method, and the potency ratio computed from ED_{50} values of aminopyrine and test compounds.

Antipyretic Activity——Five male rabbits weighing from 2.0 to 2.5 kg were used for each dose level. The rectal temperature was measured every 30 min with a portable potentiothermometer (Iio Electric Co., Ltd., Tokyo) during 1.5 hr before and 6 hr after intravenous injection of T.T.G. (Fujisawa Pharmaceutical Co., Ltd., Osaka) as a pyrogen in a dose of 10 μg/kg. Test compounds were injected intraperitoneally 1.5 hr after the injection of T.T.G. .

Anti-inflammatory Activity——The anti-inflammatory activity was tested by a rat paw edema method described by Van Arman.[8] Male Donryu rats of about 150 g body weight were used in groups of seven animals each. The rats received previously by stomach tube 5 ml of tap water in order to reduce the variability of edema production. As a phlogistic agent, 0.1 ml of 0.75% formaldehyde or 0.1 ml of 1% carrageenin solution was injected subcutaneously in right paw of rats. Test compounds were administered orally 1 hr and intraperitoneally 30 min before the injection of the phlogistic agent.

Relative potencies were estimated from the dose which produces 50% inhibition of the edema 2 hr after administration of phlogistic agent.

Acute Toxicity——The intraperitoneal and oral toxicities were determined in male ddN-JCL mice weighing between 15 and 20 g. Ten mice were used for each dose level.

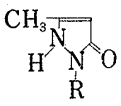
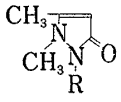
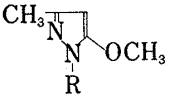
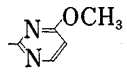
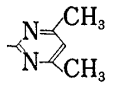
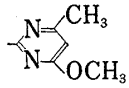
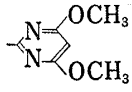
LD_{50} (72 hr) values and its fiducial limit were calculated according to the Litchfield and Wilcoxon method.[9] The compounds dissolved in distilled water or suspended in 0.5% carboxymethylcellulose were administered in a volume of 0.2 ml/10 g of body weight.

Results

The analgesic activities of test compounds determined by the pressure method are shown in Tables I, II and III.

Table I gives the relative potency of 1-(2-pyrimidinyl)pyrazole derivatives. As shown in this table, 1-(4-methoxy-6-methyl-2-pyrimidinyl)-3-methyl-5-methoxypyrazole (I) and

Table I.[a] Relative Potency of 1-(2-Pyrimidinyl)pyrazole Derivatives[b] in Analgesic Test[c] (Aminopyrine=1.00)

Formula R			
	0.59 (0.51—0.69)	0	0.73 (0.60—0.75)
	0.29 (0.22—0.32)	0.04	0.86 (0.63—1.23)
	1.36 (1.33—1.40)	0.07	2.15 (1.58—2.87)
	0.34 (0.14—0.35)	0.29 (0.14—0.37)	1.69 (0.61—3.90)

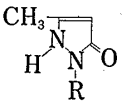
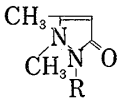
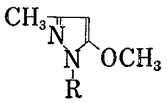
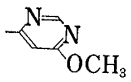
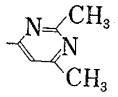
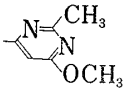
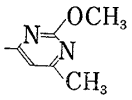
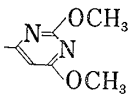
a) Figures in parentheses show the 95% fiducial limit.
b) All compounds were injected intraperitoneally.
c) Analgesic activity was measured by the pressure method in mice and relative potency of the test compound compared with aminopyrine was calculated using the four-point assay.

8) C.G. Van Arman, A.J. Begany, L.M. Miller and H.H. Pless, *J. Pharmacol. Exptl. Therap.*, **150**, 328, (1965).
9) J.T. Litchfield and F. Wilcoxon, *J. Pharmacol. Exptl. Therap.*, **96**, 99, (1949).

1-(4-methoxy-6-methyl-2-pyrimidinyl)-3-methylpyrazolin-5-one (II) were more effective than aminopyrine. N-Methylated derivatives of the pyrazolinone moiety were less effective than aminopyrine.

As regards 1-(4-pyrimidinyl)pyrazoles, as presented in Table II, 1-(6-methoxy-2-methyl-4-pyrimidinyl)-3-methylpyrazolin-5-one (III), 1-(4-pyrimidinyl)-3-methyl-5-methoxypyrazole (IV), 1-(6-methoxy-4-pyrimidinyl)-3-methyl-5-methoxypyrazole (V) and 1-(6-methoxy-2-methyl-4-pyrimidinyl)-3-methyl-5-methoxypyrazole (VI) showed almost the same or a better effect as aminopyrine. Among these compounds, N-methylated derivatives of the pyrazolinone moiety were also less effective than aminopyrine.

Table II.[a] Relative Potency of 1-(4-Pyrimidinyl)pyrazole Derivatives[b] in Analgesic Test[c] (Aminopyrine=1.00)

Formula / R			
	0.24 (0.04—0.38)	0	1.30 (1.08—1.58)
	0.23 (0.20—0.56)	0.40 (0.37—0.42)	1.41 (1.02—2.07)
	0.62 (0.60—0.64)	0.23 (0.15—0.65)	0.71 (0.55—0.95)
	1.89 (1.48—2.57)	0.63 (0.44—0.93)	1.27 (1.13—1.43)
	0.33 (0.18—0.45)	0.29	1.10 (0.90—1.36)
	0.44 (0.32—0.62)	0.88 (0.71—1.09)	0.84 (0.75—1.20)

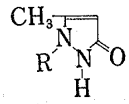
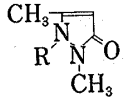
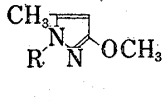
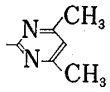
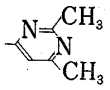
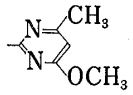
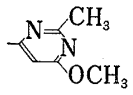
a), *b*) and *c*): same as in Table I

On the other hand, all of 2-pyrimidinylpyrazole derivatives exhibited less effect than aminopyrine as shown in Table III.

The analgesic activities of the above-mentioned six compounds proved to be equally or more active than aminopyrine. These compounds were further examined by the electric stimulation method. As presented in Table IV, the compounds which exhibited equal or better effect than aminopyrine were I, V and VI. All of these compounds are 1-pyrimidinyl-5-methoxypyrazole derivatives and have a methyl or a methoxyl group in the pyrimidine moiety. It has been found, on the whole, that the relative potencies of compounds found effective by the electric stimulation method are somewhat lower than those by the pressure method.

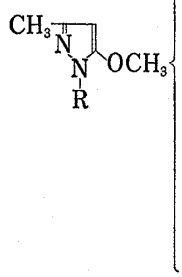
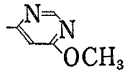
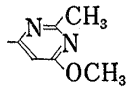
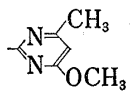
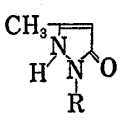
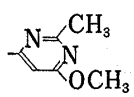
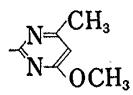
The selected compounds, I, V and VI, were subjected to the test for analgesic, antipyretic and anti-inflammatory activities, and acute toxicity. The results are summarized in Table V.

Table III.[a] Relative Potency of 2-Pyrimidinylpyrazole Derivatives[b] in Analgesic Test[c] (Aminopyrine=1.00)

Formula / R			
	0.05	—	0.33
	0.83 (0.54—1.22)	0.24 (0.18—0.34)	0.37
	0	0	0.36
	0.74 (0.47—1.01)	0	0.63 (0.46—0.80)
	0	0.35	0.31

a), *b*) and *c*): same as in Table I

Table IV.[a] Analgesic Activity of 1-Pyrimidinylpyrazole Derivatives

Compound No.	Formula	Method / R	Analgesic effect (*i.p.*) (Aminopyrine=1.00) Pressure method	Electric method
IV			1.30 (1.08—1.58)	0.61 (0.41—0.92)
V			1.41 (1.02—2.07)	1.07 (0.93—1.23)
VI			1.27 (1.13—1.43)	1.08 (1.06—1.10)
I			2.15 (1.58—2.87)	2.44 (1.72—3.46)
III			1.89 (1.48—2.57)	0.24 (0.18—0.34)
II			1.36 (1.33—1.40)	0.34 (0.28—0.42)

a) Figures in parentheses show the fiducial limit (95%) of the relative potency in analgesic test.

Analgesic effects of these three compounds administered intraperitoneally were more potent than that of aminopyrine, in both the pressure method and the electric stimulation method. Especially potent I was about twice as effective as aminopyrine. However, in the case of oral administration, I showed almost the same effect as aminopyrine, and the other two compounds, V and VI, were inferior to aminopyrine.

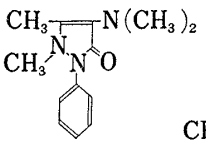
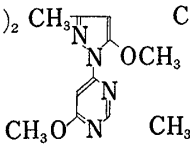
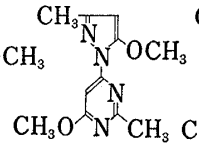
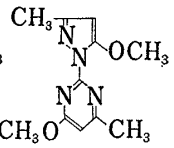
Antipyretic activities of these three compounds in rabbits were about one-half that of aminopyrine by intraperitoneal injection.

Anti-inflammatory effects of these compounds, I, V and VI, were 4 times, 2 times and 2.7 times, respectively, that of aminopyrine by the rat paw edema method using formaldehyde as the phlogistic agent.

On the other hand, the relative potencies of these compounds, I, V and VI, on carrageenin edema were 2.3, 2.9 and 2.0 by intraperitoneal injection, and 1.8, 1.1 and 1.3 by oral administration, respectively.

Acute toxicity of these compounds was lower than that of aminopyrine when injected intraperitoneally. In oral administration, I was almost equally toxic as aminopyrine, while V and VI were less toxic than aminopyrine.

Table V. Comparison of the Effects of 1-Pyrimidinylpyrazole Derivatives in Analgesic, Antipyretic, Anti-inflammatory and Acute Toxicity Tests

Method \ Test compound			Aminopyrine	V	VI	I
						
Analgesic activity[a]	Pressure method	(*i.p.*)	1.00	1.41	1.27	2.15
		(*p.o.*)	1.00	0.86	0.48	1.10
	Electric method	(*i.p.*)	1.00	1.07	1.08	2.44
		(*p.o.*)	1.00	0.86	0.18	1.06
Antipyretic activity[a]			1.00	0.5	0.5	0.5
Anti-inflammatory activity[a]	Formaldehyde	(*i.p.*)	1.00	2.0	2.7	4.0
	Carrageenin	(*i.p.*)	1.00	2.9	2.0	2.3
		(*p.o.*)	1.00	1.1	1.3	1.8
Acute toxicity[b] LD_{50} (g/kg)		(*i.p.*)	0.24	0.62	0.42	0.70
		(*p.o.*)	1.04	1.45	3.18	1.09

a) Figures show the relative potency in each method. *b*) Figures show the LD_{50} value.

Discussion

The fact that 1-pyridylpyrazolinone derivatives have analgesic, antipyretic and anti-inflammatory activities led us to investigate the pharmacological effects of a series of 1- and 2-pyrimidinylpyrazole derivatives.

Forty-four compounds in this series were subjected to the screening test for analgesic effect, and six compounds of particular interest to other pharmacological tests.

Pyrimidinylpyrazole derivatives studied were structurally divided into two classes, 1-pyrimidinylpyrazole and 2-pyrimidinylpyrazole. It is noteworthy that the six compounds found to be equal to or more potent than aminopyrine in analgesic activity all belong to 1-pyrimidinylpyrazole type, and most of them have a 5-methoxypyrazole structure. Furthermore, three of these compounds are 1-pyrimidinyl-5-methoxypyrazoles. All of 2-pyrimidinylpyrazole derivatives were less active than aminopyrine.

There are very few studies on analgesic activity of 5-methoxypyrazole derivatives. Now, the fact that 1-pyrimidinyl-5-methoxypyrazoles (I, V and VI) have a high analgesic activity

seems to suggest that the introduction of a methoxyl group into 5-position of the pyrazole moiety has an important significance in improving the activity. It should be noted, however, that I was not only about twice as effective as aminopyrine by intraperitoneal injection but also comparably effective as aminopyrine by oral administration in analgesic test.

On the other hand, the antipyretic activities of I, V and VI were relatively low compared with aminopyrine, but their anti-inflammatory activities were comparable with or more potent than aminopyrine by the paw edema method using formaldehyde and carrageenin.

Further, these compounds were found to be not more toxic than aminopyrine by the comparison of their LD_{50} values. Particularly, I was found to have a larger safety margin than aminopyrine with regard to analgesic and anti-inflammatory effects.

From these results, I may be considered as the most favorable as an analgesic and anti-inflammatory drug among the compounds studied.

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